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The successful treatment of prostate cancer requires detection of the disease at early stages. Currently the early diagnosis of prostate cancer largely depends on the detection of prostate-specific antigen (PSA) in circulation. However, PSA can only precisely detect 40% of prostate cancer and is not specific for the occurrence of prostate cancer. We reasoned that the success and accuracy in early diagnosis of prostate cancer may be significantly improved if a panel of prostate cancer-specific markers can be identified and used in combination for detecting early stage of prostate cancer. In this first year of the funding period, we focused on our efforts to construct a secretion protein gene cDNA library from early stage prostate tumor tissues. In the first seven months, we collected 24 early stage prostate tumors and prepared mRNA from these samples. We then prepared cDNA from these RNA and successfully constructed cDNA library in our pTRAP1 retroviral plasmid. In 8-14 month, we generated 10 pool of CD8-positive cells which express proteins containing secretion signals (both secreted and cell-surface proteins). In our second and third year studies, we will identify both secreted and cell surface proteins overexpressed in early stage prostate tumors.

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Table of Contents

Cover.....1

SF 298.....2

Introduction.....4

Body.....5

Key Research Accomplishments.....6

Reportable Outcomes.....6

Conclusions.....6

References..... 7

Appendices.....N/A

Introduction

The successful treatment of prostate cancer requires detection of the disease at early stages. Currently the early diagnosis of prostate cancer largely depends on the detection of prostate-specific antigen (PSA) in circulation. However, PSA can only precisely detect 40% of prostate cancer and is not specific for the occurrence of prostate cancer. We reasoned that the success and accuracy in early diagnosis of prostate cancer may be significantly improved if a panel of prostate cancer-specific markers can be identified and used in combination for detecting early stage of prostate cancer. Our proposal aims to identify a panel of secretion proteins overproduced in early stage prostate tumors. We believe that some of these proteins can be potential candidate biomarkers for the early diagnosis of prostate cancer. Through subsequent studies that are beyond the scope of this proposal, these proteins can be further analyzed for their value as early diagnostic markers for breast cancer.

Body

The goal of our research in the first year of this funding is to 1) construct a cDNA library from early stage prostate tumors in pTRAP1 retroviral plasmid (month 1-7) and to 2) collect CD8 positive cells (month 8-14). To achieve the first goal (month 1-7), we isolated mRNA from 24 early stage prostate tumors. Since the quality of mRNA was essential for generating a quality cDNA library, we performed Northern analysis to examine the integrity of the isolated RNA using β-actin as probe. As shown in Fig.1, 23 out 24 sample displayed decent β-actin expression. Equal amount of RNA from these 23 samples were pooled and subsequently subjected cDNA systhesis using Stratagene λ_BZAP cDNA synthesis kit. The resulted cDNAs were digested with Xho I and size-fractionated (cut-off 200bp). The digested cDNA were then ligated into pTRAP1 vector, and ligation reaction electroporated into *E.coli*. The bacterial were recovered in 10ml of LB for 1 hr at 37^{0}C and then propagated in 4 liter of LB. The pTRAP1 library was purified using CsCl centrifugation. Based on the number of bacterial colonies obtained after the initial transformation of ligated library DNA, we estimated that the primary library contained 1.5X10^7 independent clones. To confirm the quality of the library, 20 clones was randomly selected from the primary library and evaluated. Restriction analysis using EcoRI and XhoI revealed that 15 of these 20 clones (75%) contained cDNA inserts. From the sizes of cDNA inserts present in these clones, it was estimated that the average size of cDNA inserts in the library was approximately 450 bp. When digested with EcoRI and XhoI, cDNA inserts released from the total library DNA migrated as a smear on agarose gel, ranging from 100 bp to 2,000 bp in size. The lack of discrete bands among the cDNA species suggested that cDNA of different sizes was represented equally in this library. Based on these results, we concluded that the human prostate tumor library we had constructed was of good quality and was suitable for the subsequent secretion trap screen.

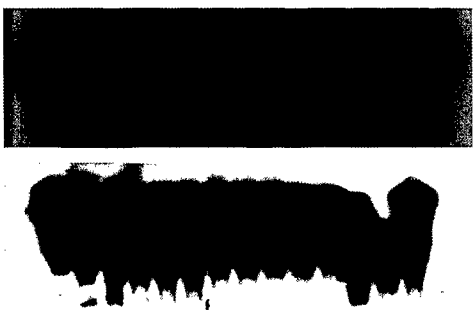

Fig. Actin mRNA expression in early stage prostate Tumors. mRNAs were isolated from 24 early stage prostate tumor samples and subjected Northern blotting to detect actin mRNA expression with labeled

To accomplish the second goal (month 8-14), we transfected pTRAP1 library into 100 dishes of Linx-A retrovirus packaging cells. The retroviruses-containing medium were collected from each dish and used to infect 100 of 10-cm plates of 293T cells (3X10^6 cells per dish) (one dish virus-containing medium used to infect a dish of 293 T cells). Based on our previous experience, retrovirus transduction efficiency on 293T cell is about 20-30%. We thus estimated that 6-9X10^7 293 cells were infected with retroviruses, and that this number is at least 4-fold redundancy of our prostate tumor library (1.5X10^7 clones). The 293 cells were selected with puromycin and the puromycin-resistant cells

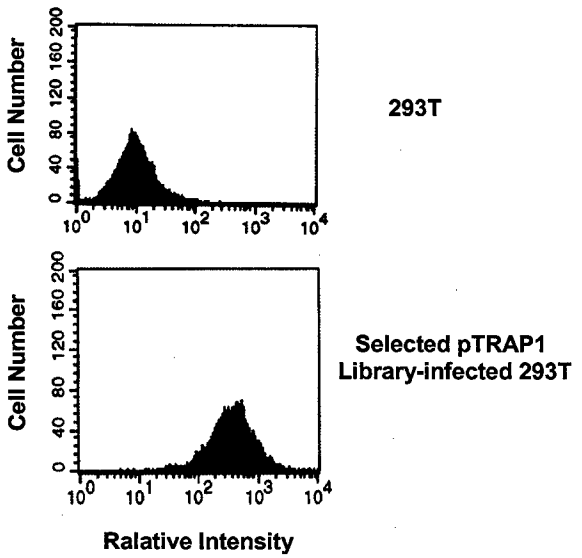

Fig.2. Puromycin-resistant pTRAP1 library infected cells were subjected to selection by CD8-dynal beads. The collected cells were combined in total 10 pools and pools. The cells from each pool was analyzed for cell surface CD8 expression by FACS. Figure shows CD8 expression in cells from pool #1. All pools showed similar patter of CD8 expression.

were incubated with Dynabeads M-250 CD8. Since all cells containing pTRAP library will express CD8 at cell surface, the library-infected cells was bound to the beads. We washed beads several times and cells bound on the beads eluted from the beads with DETACHaBEAD solution (Dynal). Cells from 10 original dishes were combined and 10 pools thus generated. Portion of each pool was analyzed by FACS for cell-surface CD8 expression using anti-CD8 mAb (Fig.2). Over 80% of cells were found to be positive for CD8 staining. Currently, we have just begun to recover provirus from each pool of CD8 positive cells and aim to generate microarray slides containing prostate tumor secretion protein cDNA library by the end of second year of the funding. So far, we are on the schedule for our milestone of this project.

Key Research Accomplishment

- We have constructed early stage prostate tumor cDNA pTRAP1 library from 23 prostate tumor samples. This has allowed us to generate 293T cells containing pTRAP1 library.
- We have finished collecting CD8-positive, pTRAP1 library-infected 293T cells. This will allow us to recover cDNAs encoding secretion proteins from prostate tumors.

Reportable Outcomes

1. A random-primed, human prostate tumor cDNA library has been constructed in the pTRAPI vector.
2. 293T cells containing prostate tumor cDNA library has been collected.

Conclusions

We have generated early stage prostate tumor cDNA library in pTRAP1 vector system and cells containing this library. With continued proposed studies in second and third year, we hope that we can identify proteins overexpressed in early stage prostate cancer.

References

None

Appendices

N/A